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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 3: A61B 10/00; A61K 9/50; A61B 5/02 — A1

(11) International Publication Number: WO 80/02365

(43) International Publication Date: 13 November 1980 (13.11.80)

(21) International Application Number: PCT/US80/00502

(22) International Filing Date: 2 May 1980 (02.05.80)

(31) Priority Application Number: 036,098

(32) Priority Date: 4 May 1979 (04.05.79)

(33) Priority Country: US

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(81) Designated States: AT (European patent), AU, CH (European patent), DE (European patent), FR (European patent), GB (European patent), JP, NL (European patent), SE (European patent).

Published

With international search report

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ULTRASONIC IMAGE ENHANCEMENT

(57) Abstract

Ultrasonic images of flowing streams can provide important information regarding the streams. Herein, a plurality of microbubbles are provided in such streams to enhance such images, aid in tumor detection and treatment, provide maping of vascularity of tissue masses and measure instantaneous blood flow rate. The preferred microbubbles have a coalescence resistant surface membrane encapsulating a gas of a selected composition, the membrane including non-toxic and non-antigenic organic molecules. Preferably, the microbubbles have diameters in the 0.5 micron to 300 microns range.

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Description

Ultrasonic Image Enhancement

Technicol Field

This invention relates to an ultrasonic image enhancement method, to diagnostic techniques and to treatment methods which are closely related thereto.

Background Art

Contrast agents are often employed medically to accentuate subtle differences between two structures in X-ray radiographic images. In X-ray diagnosis, for example, a radiopaque dye is rountinely injected into an arterial bed to delineate the existing vasculature which otherwise could not be detected. Present ultrasonic diagnosis generally faces similar problems. The ultrasonographer has comparable difficulty in detecting certain structures, for example septal defects in small children, but no effective ultrasonic contrast agent has been available. An acceptable ultrasonic contrast agent which can be delivered into the blood stream therefore is greatly needed. A selective agent, i.e., one which can selectively emphasize particular parts of the vasculature (such as that of a tumor), would be especially valuable.

Measurement of cardiac output and other quantitative blood flow measurements are needed to monitor the health of many patients. Existing non-invasive measurement techniques are indirect and only approximate. Existing reliable and accurate measurement techniques involve catheterization, a

It would be desirable to provide a method of controllably and uniformly enhancing ultrasonic images of the blood stream of a living subject. Such a method could be utilized, for example, for detecting tumors and other abnormalities, for measuring instantaneous cardiac output and flow velocities in other vessels, for delivering gaseous therapeutic agents selectively to tumors or other tissues, and the like.

Disclosure of Invention

The present invention is directed toward overcoming one or more of the shortcomings of the prior art as set forth above.

According to the present invention, a method is set out for enhancing ultrasonic images of the blood stream of a patient. The method comprises flowing a plurality of microbubbles, each having a surface membrane encapsulating a gas of a selected composition, the membrane including a multiplicity of non-toxic and non-antigenic organic molecules, the microbubbles each having a diameter of no more than about 300 microns and no less than about 0.5 microns, in the blood stream; obtaining ultrasonic images of the blood stream opposite a position therein through which the microbubbles are flowing, thereby rendering the blood-carrying vessel visible by virtue of the increased contrast of the bloodstream from the surrounding tissue, and permitting detection of abnormalities in configuration or function of the vessel.

In another sense, the invention comprises a method of measuring instantaneous flow in blood vessels including cardiac output. The method

comprises injecting a substance into a blood stream of a test subject, the substance providing an ensemble of microbubbles; measuring the instantaneous velocities of the microbubbles at a location in the blood stream by substantially simultaneously measuring the time dependent positions of the ensemble of microbubbles across the diameter of the vessel at said location; and determining therefrom the substantially instantaneous volumetric flow rate at the location in the blood stream.

In still another sense, the invention relates to a method of detecting tumors in a living subject. The method comprises injecting a substance into a blood stream of the subject, the substance providing a plurality of controlled size microbubbles in the blood stream; obtaining an ultrasonic image of the bubbles; and examining the image for evidences of neovascularization, with or without a necrotic core, indicative of a possible tumor.

Still further, in another embodiment of the invention, a method is provided of delivering a gaseous therapeutic agent selectively to tumorous tissue. The method comprises injecting such microbubbles as have been previously discussed, wherein the gas therein comprises a therapeutic agent.

In yet another sense, the invention comprises a method of measuring the afferent vascularity of a certain tissue mass. The method comprises injecting or infusing a substance providing a plurality of precision microbubbles. The bubble diameter is preselected for the dimension of concern. The microbubbles flow into the general area for ultrasonic examination and lodge at a bifurcation whose discharge branches are all smaller than the

in certain instances it may be desirable to utilize a gas which is far from inert. For example, it may be desirable to utilize a gas within the bubble which is toxic to tissue, if the bubble is designed to be absorbed by tumorous tissue but to not be absorbed by the normal tissue of the blood stream. In other instances, it may be desirable to employ a gas which dissolves in blood quickly, such as carbon dioxide.

Another important result of utilizing microbubbles having the particular membrane described above is that they will have a reduced tendency to stick to the walls of the blood vessel, particularly the walls of normal blood vessels. With tumorous tissue, the walls of the blood vessels are considerably rougher and otherwise abnormal, thus providing a more ready accepting surface for holding such microbubbles, even with their reduced tendency to stick to normal blood vessel walls.

The size of such microbubbles is also important. Generally they will be, at most, about 300 microns, and at least about 0.5 micron, in diameter. More preferably, the microbubbles will have a diameter below about 150 microns and above about 1.0 micron. In some instances, all of the microbubbles injected will be of about the same size so that they congregate in a particular area of the body or in a particular type or size of blood vessel. Microbubbles between 5 and 10 microns are particularly useful in that they can pass through normal capillaries.

Such microbubbles as have just been described are produced by gradually flowing a gas through a small orifice, for example through a capillary tube, and into a liquid. A force is

generally exerted upon the microbubble being formed at the orifice, with the force being sufficient to remove the microbubble prior to its attaining the full size it would attain in the absence of such force. For example, the orifice may lie generally in a vertical plane (the capillary may be horizontal) and the force may simply comprise the buoyancy of the microbubble in the liquid and the surface tension attachment to the orifice. Alternately, and preferably, the orifice may lie in any orientation with flow past the orifice, and the force consists of fluid drag on the bubble and the surface tension force. In both situations the microbubbles may flow into a storage container such as a hypodermic syringe. The aforementioned report "Non-Invasive Assessment of Pulmonary Hypertension Using The Bubble Ultrasonic Resonance Pressure (BURP) Method" describes production of such microbubbles in more detail.

Other methods of producing the described microbubbles have been successfully employed. For example, microbubbles have been created by supersaturation of a liquid; air or liquid jet impingement upon a free liquid surface; and addition of $NaHCO_3$ particles to a liquid. These latter methods permit production of large quantities of microbubbles but of a much broader spectrum of sizes than the highly uniform diameter of microbubbles produced by a submerged orifice.

It is preferred that the microbubbles be formed and dispersed in a medium having a chemical composition substantially identical to that of the membrane. It is further preferred that the medium be gellable. As previously mentioned, a particularly preferred membrane material is gelatin itself, because

abnormalities such as septal defects and valvular disfunctions in the heart, and obstructions or aneurysms in the coronary, aorta, carotoid and other vital blood vessels. Furthermore, by integrating the velocity profile across the diameter of a great vessel of the heart such as the pulmonary artery or the ascending aorta, a direct measurement of instantaneous cardiac output can be obtained non-invasively. This important measurement previously could be obtained only by catheterization, a difficult and hazardous procedure, or by inaccurate and ambiguous indirect methods, and as averages over the cardiac cycle rather than instantaneous values.

Tumors can be detected in a living test subject via ultrasonic images by observing the abnormal concentration of microbubbles in an area where a tumor is suspected. Basically, the vasculature of a tumor grows at a rapid rate and becomes erratic and larger than that of normal tissue. Because the neovascularized vessels are larger than normal vessels, increased blood flow exists and a much higher concentration of microbubbles will be present. In particular, if microbubbles of a particularly appropriate size are chosen, it is possible to selectively collect such microbubbles within the tumor neovasculature and to thus delineate the extent of the neovascularity with a quantitative image. Alternately, by using microbubbles of a uniform size larger than the normal capillary diameter, i.e., 7 to 10 microns, but well within the range of abnormal tumor capillary diameter, i.e., 20 to 100 microns, the local presence of the tumor is unambiguously identified by microbubbles which pass through the afferent vasculature and appear in the

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efferent (venous) vasculature.

Example

The following example illustrates the use of microbubbles as ultrasonic contrast agents.

Nitrogen microbubbles (38, 80 and 140 microns in diameter) dispersed in gelatin, were injected via a catheter into test subjects. Static (5mHz transducer) and real time (7.5mHz transducer) images were recorded on Polaroid film and on videotape. Rabbits with unilateral thigh V2 carcinomas were used in the in vivo studies. Baseline ultrasound images of normal muscle and V2 carcinoma were obtained. Five milliliter syringes containing a gelatin dispersion of 80 micron nitrogen microbubbles were warmed and injected through a catheter placed in the V2 ipsilateral iliac artery via the right carotid artery. Static and real time images of normal muscle, blood vessels, and the V2 carcinoma, which was located by palpation, were recorded for at least 2 minutes following each injection. The gelatin-encapsulated nitrogen bubbles were also readily demonstrated in an in vitro phantom. The 80 and 140 micron bubbles were more echogenic that the 38 micron bubbles, although this may be a result of the instrumentation and geometry of the test. In vivo, the 80 micron microbubbles could be identified for several minutes after the initial bolus of bubbles. The central anechoic portions of the V2 carcinoma did not become echogenic following injection of microbubbles but the periphery of the tumor became increasingly echogenic. The gelatin-encapsulated nitrogen microbubbles are thus demonstrated as being an effective ultrasonic contrast agent. The ultrasonic tumor rim enhancement

6. A method of detecting tumors in a living subject, comprising:

injecting a substance into a blood stream of said test subject, said substance providing a plurality of microbubbles in said blood stream;

obtaining an ultrasonic image of said bubbles; and

examining said image for evidence of neovascularization indicative of a possible tumor.

7. A method as in claim 6, wherein said substance comprises a plurality of microbubbles each having a surface membrane encapsulating a gas of a selected composition, said membrane including a multiplicity of non-toxic and non-antigenic organic molecules.

8. A method as in claim 7, wherein said molecules have a hydrophilic portion and a hydrophobic portion, said hydrophilic portions being aligned radially away from a center of each respective bubble, said microbubbles are of generally a uniform size and of a diameter of no more than about 300 microns and no less than about 0.5 micron.

9. A method as in claim 7, wherein said membrane is of a gelable composition.

10. A method as in claim 7, wherein said membrane comprises gelatin.

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11. A method of delivering a gaseous therapeutic agent selectively to tumorous tissue, comprising:

injecting a plurality of microbubbles into a blood stream of a living subject, each microbubble having a surface membrane encapsulating a gas of a selected composition, said membrane including a multiplicity of non-toxic and non-antigenic organic molecules, said microbubbles having a diameter of no more than about 300 microns and no less than about 0.5 micron, said gas comprising a therapeutic agent.

12. A method as in claim 11, wherein said microbubbles are of generally a uniform size.

13. A method as in claim 12, wherein said molecules have a hydrophilic portion and a hydrophobic portion, said hydrophilic portions being aligned radially away from a center of each respective bubble, said microbubbles being of generally a uniform size.

14. A method as in claim 12, wherein said membrane is of a gelable composition.

15. A method as in claim 12, wherein said membrane comprises gelatin.

24. A method as in claim 22, wherein said membrane is of a gelable composition.

25. A method as in claim 24, wherein said gelable composition is gelatin.

26. A method of detecting tumorous tissue in a living subject, comprising:

injecting a substance into an afferent vasculature of said test subject upstream of a possible tumor, said substance providing a plurality of microbubbles in said blood stream of a diameter too large to pass through normal capillaries but small enough to pass through tumorous capillaries; and

obtaining an ultrasonic image of a corresponding efferent vasculature downstream of said possible tumor and noting if such microbubbles are present.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US80/00502

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL.³ A61B 10/00, A61K9/50, A61B5/02
U.S. CL. 128/660 424/19, 128/663

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System	Classification Symbols
U.S.	128/660,661,662,663 73/194A,861.05,861.06 424/4.13,19 252/316

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched †

III. DOCUMENTS CONSIDERED TO BE RELEVANT **

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages ††	Relevant to Claim No. ††
X,P	US, A, 4,182,173 Published 08 JANUARY 1980 Papadofrangakis et al	1-10, 22-26
X	IEEE TRANSACTION ON Biomedical Engineering, Vol. BME-24, No. 2, MARCH 1977, Fairbank, W.M. et al, " A New Non-Invasive Technique For Cardiac Pressure Measurement; Resonant Scattering of Ultrasound from Bubbles", pages 107-110	1-10, 22-26
X	Report No. HR-62917-1A prepared for National Heart, Lung and Blood Institute, Bethesda, Maryland, APRIL 1977, Tickner, E.G. et al "Non-Invasive Assessment of Pulmonary Hypertension Using the Bubble UTS Resonance Pressure (BURP) Method"	1-10, 22-26
A	US, A, 3,640,271 Published 02 FEBRUARY 1972 Horton	
A	Journal of Biomechanics, Vol.4,1971, Yang, W. et al. "Experimental Studies of Dissolution of Gas Bubbles in Whole Blood and Plasma", pages 275-281	

* Special categories of cited documents: ††

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IV. CERTIFICATION

Date of the Actual Completion of the International Search *	Date of Mailing of this International Search Report *
Search may be extended if Form 206 fees paid	11 SEP 1980
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Form PCT/ISA/210 (second sheet) (October 1977)